# Myclobutanil Risk Assessment

Division of Pest Management, Environmental Protection, and Worker Safety

California Department of Food and Agriculture

August 16, 1988

## I. Summary

Myclobutanil is a systemic fungicide which is the active ingredient in formulated products to be sold under the name Rally. Possible adverse effects have been identified in rat chronic and developmental toxicity studies. In the chronic study, the effect was testicular atrophy, at a daily dosage of 10 mg/kg with a NOAEL of 2.5 mg/kg-day. In the rat teratology study, fetal viability was decreased in the 94 mg/kg-day group, with a NOAEL of 31 mg/kg-day. Margins of safety for current uses are at least 625, based on worker exposure studies submitted by the registrant, and dietary exposure calculations.

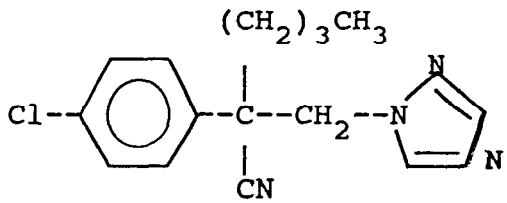
## II. Introduction

### A. Identification and Usage

Rally 40W is a systemic fungicide product containing 39.5% myclobutanil (alpha-butyl-alpha-(4-chlorophenyl)-1H-1,2,4-triazole-1-propanenitrile), for which a California experimental use permit is pending. Its mode of fungicidal action is inhibition of ergosterol biosynthesis. There is one registrant and one formulation. The only currently proposed use is on grapes to control powdery mildew and black rot. It is applied at rates of 0.075 to 0.125 lbs. a.i. (3-5 oz. Rally 40W) per acre in at least 50 gallons of water. Multiple applications are proposed at 14 - 21 day intervals up to a maximum of 1.5 lbs Rally 40W per acre. Pre-harvest interval is 14 days.

### B. Physical/chemical properties of myclobutanil (1)

1. Empirical formula: $C_{15}H_{17}Cl\ N_4$
2. Molecular weight 288.8
3. Melting point 63-68°C
4. Boiling point 202-208°C
5. Stability Stable up to 300°C
6. Solubility 142 ppm in water @ 25°C, soluble in common organic solvents except aliphatic hydrocarbons
7. Appearance light yellow solid
8. Octanol/Water partition coefficient: 871 @ 25°C
9. Vapor Pressure $1.6 \times 10^{-6}$ torr @ 25°C
10. Structural Formula



## III. Toxicological Profile

### A. Acute Toxicity

The oral median lethal dose of myclobutanil 91.9% technical in male rats is 1600 mg/kg, and in females, 2290 mg/kg (2). The oral median lethal dosage of a newer 84.5% technical is 1750 (males) and 1800 (females) (2a). Signs of intoxication included ataxia, passiveness, prostration, salivation, lacrimation, stained muzzle, and alopecia. Necropsy findings included enlarged stomach with reddened gastric and intestinal mucosa, and reddened lungs.

The dermal median lethal dose in rabbits is >5000 mg/kg for both the 91.9% technical and the 84.5% technical (3,3a). Signs in intoxicated animals included passiveness, ataxia, and alopecia, with affected animals showing an irregular granular surface of the liver at necropsy.

The median lethal concentration for a 4-hour exposure is >5 mg myclobutanil 91.4% technical per liter of air. Clinical observations in exposed animals included bradypnea, dyspnea, rales, gasping, nasal exudate, unthriftyness, and transient decreases in food consumption and fecal output (4). No significant lesions were seen at necropsy.

Myclobutanil 91.9% technical is a category I eye irritant, a category IV skin irritant, and a weak contact sensitizer (5,6,6a). Myclobutanil 84.5% technical is a category IV skin and eye irritant (6b,6c).

Rally 40WP (39.5% a.i.) has an oral $LD_{50}$ of 1870 (male) to 2090 (female) mg/kg in rats and a dermal $LD_{50}$ >5000 mg/kg in rabbits (7,8). The median lethal concentration for rats is >5000 mg of the dust per liter of air (analytical concentration) (9). Rally 40WP is a category III eye irritant and a category IV skin irritant (10,11). It was not a dermal sensitizer (12).

Clothing requirements indicated on the label include long trousers, long-sleeved shirt, impervious gloves, hat, and splash goggles during mixing, loading, applying, or handling. The signal word for the Rally 40W is "Warning", although the acute toxicity data indicate "Caution". Re-entry is permitted after the spray has dried.

### B. Metabolism and Pharmacokinetics

Following an oral gavage dose of 150 mg/kg, myclobutanil was rapidly and completely absorbed from the gut of rats, with a half-life of about 0.2 hr. Elimination is also rapid, equally divided between urine and feces. The half-life in plasma and tissues is 2-5 hr. initially, followed by a slower phase with a half-life of 25 hr. Intravenous or oral doses were similarly excreted, indicatng that the oral dose had been completely absorbed (13). Parent material was less than 4% of the excreted radiolabel, and the metabolites were more polar. Nine major components were excreted, which involve oxidation of the butyl group; some are also sulfated. Glucuronides were not found (14,15). Two of the major animal metabolites, RH-9090 and RH-9089 are oxidized on the butyl sidechain. They are also the major metabolites in grapes, apples and wheat, and, along with RH-9090 glycoside and the parent compound, account for virtually 100% of recovered residues.

C. Sub-chronic Toxicity

In a 3-month rat feeding study, the NOAEL was 1000 ppm (50 mg of an 81.1% technical/kg/day), based on histological changes in the liver, kidney, and adrenals, and clinical pathological changes related to liver and kidney function at dietary concentrations of 3000 and 10,000 ppm. No rats fed diets containing 30,000 ppm survived beyond 63 days (16).

In a 3-month dog feeding study, the NOAEL was 1600 ppm (48 mg of an 81.1% technical/kg/day), HDT. Liver hypertrophy and increased serum alkaline phosphatase were reported in the 800 and 1600 ppm groups (17). Similar changes were reported in the 1-year dog study, at slightly lower dosages.

Rats treated daily for four weeks with topical applications of up to 100 mg/kg of Rally 40WP or 2EC exhibited no systemic effects (19).

D. Chronic Toxicity/Oncogenicity

Myclobutanil technical (2 lots, 90.4 & 91.4% pure) was fed to 110 Sprague-Dawley rats/sex/group (50-58/group for interim sacrifice) at dietary concentrations of 0, 50, 200 or 800 ppm for 2 years (20). Testicular atrophy, characterized by seminiferous tubules devoid of germinal epithelium and spermatid precursors, was seen up to twice as frequently in the 200 and 800 ppm males as in the controls, giving a NOAEL of 50 ppm (2.5 mg/kg-day). Relative liver weights were in increased in 800 ppm females. No oncogenic effect was noted. Hepatocellular hypertrophy, observed at dosages of 200 and 1000 ppm in the reproduction study discussed in the following section, was not seen in this study.

Myclobutanil technical (91.4% purity) was fed to Beagle dogs (6/sex/group) at dietary concentrations of 0, 10, 100, 400 or 1600 ppm for 1 year. Mild liver effects, characterized by hepatocellular hypertrophy (both sexes), increases in serum alanine aminotransferase (males), gamma glutamyl transpeptidase and serum alkaline phosphatase levels (females) were identified at a dosage of 400 ppm (~12 mg/kg-day), with a NOEL of 100 ppm (~3 mg/kg-day) (21).

Myclobutanil technical (lot LAP-0298, 90.4% pure) was fed to 70 COBS CD-1 mice/sex/group at dietary concentrations of 0, 20, 100 or 500 ppm for 2 years (with additional mice for interim sacrifice)(22). There was no oncogenic response. Based on increased hepatic mixed function oxidase activity at 100 ppm (~15 mg/kg-day), particularly in females, the NOEL was 20 ppm (~3 mg/kg-day). Degenerative hepatocellular changes were reported in 500 ppm males, but not at 100 ppm (~15 mg/kg-day). Similar changes were seen in a 3-month mouse feeding study using a 81.1% technical, at dietary concentrations of 1000 ppm in males and 3000 ppm in females (18).

F. Developmental and Reproductive Toxicology

Myclobutanil technical (84.5% pure) was fed to 2 generations of rats (25/sex/group) at dietary concentrations 0, 50, 200 or 1000 ppm for 8 weeks before mating and through two mating, gestation, and lactation periods. The NOEL for general toxicity was 50 ppm, based on centrolobular hepatocyte hypertrophy in 200 ppm males and more generalized hepatotoxicity in both sexes fed 1000 ppm. The no-reproductive-effect level was 200 ppm (~8.5 mg a.i./kg-day), based on testicular and prostatic atrophy, reduced pregnancy rate (associated with male lesions noted above), slightly increased incidence of stillbirths, and retarded growth of pups during lactation in the 1000 ppm group. Similar male reproductive lesions were seen in rats fed diets containing 200 ppm in the 2-year study, which therefore becomes limiting. The relationship between the functional and anatomical reproductive effects and the liver lesions is uncertain, but testicular atrophy occurred in the chronic study in the absence of hepatotoxicity.

Myclobutanil technical (lot #83-0017E, 84.5% a.i.) was administered by gavage to 25 Sprague-Dawley rats/group on days 6-15 of gestation at dosages of 0, 31.3, 93.8, 312.6, or 468.9 mg/kg. The maternal toxicity NOEL was 93.8 mg/kg, based on increased incidence of clinical observations. The developmental toxicity NOEL was 31.3 mg/kg, based on decreased viability index at 93.8 and increased incidence of skeletal variants at 312.6 and above. These are considered adverse effects, since developmental toxicity occurred at a non-maternally-toxic dosage (24).

Myclobutanil technical (lot #LAP-0298, 90.4% purity) was given to 18 NZW rabbits/group at dosages of 0, 0 (vehicle & water), 20, 60 or 200 mg/kg oral gavage days 7-19 of gestation. The maternal toxicity NOEL was 20 mg/kg, based on reduced weight gain. Increased resorptions were observed at a dosage of 200 mg/kg-day, giving a developmental toxicity NOEL of 60 mg/kg. No teratogenic effect was observed (25).

The plant metabolite triazole alanine (Batch #TLB 1207/018-024 97.6%, from Bayer AG) was fed to 15 males and 30 females per group at dietary concentrations of 0, 500, 2000 or 10,000 ppm for 2 generations, 2 litters per generation. Analysis of diets over the study indicated the mean for 10,000 ppm to be 9586 ppm. The reproduction NOEL was 2000 ppm based on slightly lower pup weight at day 1 in F1B, F2A and B litters at high dose. These were not considered adverse effects, so the NOAEL was 10,000 ppm (26).

G. Genetic Toxicology

With satisfactory studies in all three categories of genotoxicity testing, no adverse effects have been identified:

Myclobutanil (lot #RPO8154-5, >99% pure) was tested for gene mutagenicity with Salmonella typhimurium strains TA1535, TA1537, TA98 and TA100 with and without rat liver activation by plate incorporation assay. Concentrations were 0, 75, 250, 500, 750, 1000, 1500, 2500, 5000, and 7500 ug/plate, with five per strain. Colony counts were decreased at higher concentrations in all strains, with no increase in reversion rate reported. The study was unacceptable because of an inadequate protocol, no individual plate counts, no indication of the number of plates per concentration, missing positive controls without activation (27).

Myclobutanil (lot LSPL83-0017E, 84.5% with 6.5% RH-964) was tested with Salmonella typhimurium strains TA1535, TA1537, TA98 and TA100 by plate incorporation, with and without rat liver activation, at concentrations of 0, 75, 250, 500, 750, 1000, 1500, 2500, and 7500 g/plate. The number of plates per concentration was not given. No increase in reversion rate was reported. The study was unacceptable due to an inadequate protocol, no individual plates, and no positive controls without activation (28).

:butanil (90.4% technical, Lot LAP-0298) was tested with S9 from rat _ _ _ _ at concentrations of 0, 75, 250, 750, 2500 or 7500 ug/plate in triplicate. There was cytotoxicity at high concentrations, but no evidence for mutagenicity. The study was unacceptable (single trial) (29).

Myclobutanil technical (81.%) and Rally 40W were tested at concentrations of 0, 25, 60, 80, 85 or 90 ug/ml, 18-20 hours without activation, and at 0, 120, 150, 155, 160 170 or 175 ug/ml, 5 hours with rat liver S9 activation, using an 8-day expression. No mutagenic effect was reported (30).

Triazole alanine technical (97.4%) was tested with strains TA1535, TA1537, TA98, TA100 and TA102 with and without rat liver activation, at concentrations of 0, 20, 78, 313, 1250 or 5000 ug/plate, triplicate plates, two trials. There was no evidence for an increase in reversion rate. Not acceptable to fill data gap for parent compound but performed in an acceptable manner (31).

Triazolylalanine (97.4%) tested with and without activation at 0, 500, 1000, 2000, 4000, 6000, 8000 or 10,000 ug/ml; 21 hours -S9, 5 hours +S9; two trials; no increase in mutation frequency; complete but not acceptable for data gap for parental compound. Study was, however, conducted in an acceptable manner (32).

Myclobutanil (91.4%) was administered by gavage to 25 male rats/group at dosages of 0, 10, 100 or 735 mg/kg. Each was mated with 2 females for 8 weekly periods. No dominant lethal effect was apparent. The study was unacceptable (no positive control data) (33).

Myclobutanil technical (81.5%) given orally in a single dose of 0, 65, 260 or 650 mg a.i./kg to 30 male mice per group with sacrifice at 6, 24 & 48 hours of 10/group, and 5 daily doses to 10 males/group with sacrifice 6 hours after 2nd dosing, resulted in no increased incidence of aberrations. Unacceptable (use of males only, no evidence for MTD or marrow toxicity (34).

Myclobutanil technical (lot #83159, 91.9% a.i.) was tested in duplicate CHO cultures at 20, 30, 40 or 50 ug/ml with activation incubated for 2 hours or at 25, 50 or 75 ug/ml without activation for 17.5 hours. No increase in chromosome aberrations was reported (35).

Myclobutanil (91.4%) was given by gavage at dosages of 0, 117, 585 or 1170 mg ai/kg (corrected for purity). Groups of 7 mice/sex were sacrificed at 6, 27 and 51 hours after treatment with metaphase spreads from 5/sex in the high dose groups being scored, 50 spreads per animal. TEM was used as a positive control. The high dosage was approximately the LD10. No increase in chromosomal aberrations was reported in the high dose group (36).

Triazole alanine (97.4%) given by gavage to 24/sex at 0 or 5000 mg/kg with sacrifice of 8/sex/group at 16, 24 and 48 hours resulted in no reported increase in micronuclei. The study was unacceptable (dose selection - no evidence of MTD or bone marrow cytotoxicity) (37).

Myclobutanil technical (lot #83159-5 91.9%) was tested with rat hepatocytes at concentrations of 0, 0.5, 1.0, 5.0 or 10.0 ug/ml, with 50 cells from each of 3 coverslips scored. No net increase in grain counts was reported (38).

Triazole alanine (97.4%) tested with primary rat hepatocytes at 0, 0.08. 0.4, 2 or 10 mg/ml for 5 hours with 150 cells counted from each of three slides per concentration, showed no evidence for unscheduled DNA synthesis (39).

## IV. Risk Assessment

### A. Hazard Identification

Possible adverse effects have been identified in myclobutanil rat chronic and developmental toxicity studies. In the rat chronic study the effect was testicular atrophy, with a LOAEL of 200 ppm (10 mg/kg-day), and a NOAEL of 50 ppm (2.5 mg/kg-day). No comparable testicular lesions were observed in the chronic mouse or dog studies, but in the rat reproduction study, testicular atrophy occurred in the second generation in the 1000 ppm group (but not in the 200 ppm group), with a marked correlation between this lesion and failure to sire offspring. The developmental effect in rats was a decreased viability in the 94 mg/kg-day group and increased skeletal variants in the 313 mg/kg-day group, giving a NOAEL of 31 mg/kg/day. Despite a structural similarity to some goitrogenic herbicides, neither structural thyroid abnormalities nor clinical changes that could be related to abnormal thyroid function were identified. Acute lethal dosages are at least 500 times the lowest no-effect level.

### B. Exposure Assessment

A mixer/loader/applicator exposure study was conducted in conjunction with six vineyard applications. Exposure was monitored by dermal dosimeters, dermal swabbing, respirators, and urine monitoring. Even though the latter method failed to detect any residues of myclobutanil or its principal metabolite, dosages were estimated based on the other methods, assuming 100% dermal absorption (respiratory exposure was negligible; details in Appendix B):

| | Exposure (mg/12-hr) | Daily[1] Dosage (ug/kg) | Developmental[2] Toxicity MOS | Annual[3] Workdays | AADD[4] (ug/kg) | Chronic[5] Toxicity MOS |
|---|---|---|---|---|---|---|
| Applicator | | | | | | |
| Unprotected[6] | 0.36 | 6.6 | 4700 | 40 | 0.73 | 3425 |
| Protected[7] | 0.01 | 0.2 | 155000 | 40 | 0.02 | 125000 |
| Mixer/loader | | | | | | |
| Unprotected | 0.10 | 1.9 | 16320 | 40 | 0.21 | 11900 |
| Protected | 0.02 | 0.4 | 77500 | 40 | 0.04 | 62500 |
| Mixer/loader/Applicator | | | | | | |
| Unprotected | 0.47 | 8.5 | 3650 | 40 | 0.93 | 2690 |
| Protected | 0.03 | 0.6 | 52170 | 40 | 0.07 | 35710 |
| Re-entry[8] | 1.4 | 26 | 1190 | 60 | 4 | 625 |
| Dietary, maximum[9] | | | | | 1.9 | 1300 |

1. Dermal exposure x 100% absorption ÷ 54.8 kg body weight
2. NOEL for developmental toxicity i.e. 31 mg/kg-day ÷ daily dosage
3. Estimated annual work days for this activity
4. Annual Average Daily Dosage = daily dosage x fraction of days worked.
5. NOEL for testicular atrophy i.e. 2.5 mg/kg-day ÷ AADD x 70 kg/54.8 kg
6. Short sleeves, no hat or gloves
7. Long sleeves, hat, and gloves
8. Maximum estimate at 2 hours post-application, using a transfer coefficient of 10000 $cm^2$/hr; (see appendix B for details)
9. Based on non-nursing infants, assuming that all residues are present at the tolerance. Other groups ranged from 0.3 to 1.5 ug/kg-day.

C. Quantitative Risk Assessment/Risk Characterization

For all current exposure estimates, safety margins for developmental toxicity and testicular atrophy are adequate to allow for interspecies and intraspecies extrapolation. Safety margins for acute lethality are at least 400,000. Since projected exposures at re-entry are 1/625 of the NOEL for developmental and reproductive toxicity, warnings under the Safe Drinking Water and Toxic Enforcement Act of 1986 will be required, if the 1000-fold safety factor is imposed. If the latter requirement is considered flexibly, we would recommend that the margin of safety of 625 for myclobutanil-induced reproductive effects is adequate, since the effect at the 10 mg/kg-day LOEL were marginal, and not repeated in the reproduction study, and the ratio of this dosage to the maximum estimated exposure is 2500. All other estimated exposures are less than 1/1000 of the NOEL.

## V. Risk Management

Margins of safety for current uses are adequate, based on worker exposure studies submitted by the registrant, and on dietary exposures estimated using the TAS system and tolerances in place of residue data. Future additional uses and exposures (e.g. additional foods or drinking water) will need to be evaluated for projected impact on total exposure. Annual average daily dosages limited to 25 ug/kg would provide a 100-fold margin of safety for male reproductive effects.

## VI. Conclusions

Myclobutanil is a systemic fungicide which is the active ingredient in formulated products to be sold under the name Rally. Possible adverse effects have been identified in rat chronic and developmental toxicity studies. In the chronic study, the effect was testicular atrophy, with a LOEL of 10 mg/kg-day, and a NOAEL of approximately 2.5 mg/kg-day. In the rat teratology study, decreased viability was observed in the 94 mg/kg-day group, with a NOAEL of 31 mg/kg-day. While future additional use patterns and exposure routes will need to be evaluated for projected impact on total exposure, margins of safety for current uses are at least 635 for chronic toxicity and at least 1190 for reproductive effects.

## VII. References


1. Farm Chemicals Handbook, 1988 edition.

2. Rohm & Haas #84R-063 A/B, 1984, RH-53,866: Acute Oral $LD_{50}$ Definitive, Male (Female) Rats - Compound RH-53,866 Technical; V. 51315-099, #52512

2a. Rohm & Haas #83P-201 A/B, 1983, RH-53,866: Acute Oral $LD_{50}$ Definitive, Rats (Male, Female) - Myclobutanil 84.5% Technical; V. 51315-104, #64984

3. Rohm & Haas,#84R-134A/B, 1984, Acute Definitive Dermal $LD_{50}$ in Rabbits, Compound RH-53,866 technical V. 51315-099 #52513

3a. Rohm & Haas,#83P-194, 1983, Acute Definitive Dermal $LD_{50}$ in Rabbits, Myclobutanil 84.5% Technical; V. 51315-104, #64985

4. Rohm & Haas #87R-028, 1987, RH-3866 Technical Acute Inhalation Toxicity Study in Rats; V. 51315-104 #65088

5. Rohm & Haas #84R-134A, 1984; Acute Definitive Eye Irritation Study in Rabbits Compound RH-53,866 technical; V.51315 099 #52515

Rohm & Haas #84R-134A, 1984; RH-53,866: Acute Definitive Skin Irritation Study in Rabbits; V. 51315-099 #52514

6a. Rohm & Haas #87R-035A, 1984; RH-3,866: Delayed Contact Hypersensitivity Study in Guinea Pigs; V. 51315-104 #64994

6b. Rohm & Haas #83P-192, 1983; Acute Definitive Eye Irritation Study in Rabbits; Myclobutanil 84.5% technical; V.51315-104 #64986

6c. Rohm & Haas #83P-191, 1983; Acute Definitive Skin Irritation Study in Rabbits, Myclobutanil 84.5% technical; V. 51315-104 #64987

6. Rohm & Haas #84R-134A, 1984; RH-53,866: Acute Definitive Skin Irritation Study in Rabbits; V. 51315-099 #52514

7. Rohm & Haas #84R-082A/B, 1984; RH-53,866: Acute Oral Toxicity Study in Rats; V. 51315-048 #51845

8. Rohm & Haas #84R-082A/B) 1984; RH-53,866: Acute Dermal Toxicity Study in Rabbits; V. 51315-048 #51846

9. Rohm & Haas #84R-047, 1984, RH-53,866 40WP: Acute Dust Inhalation Toxicity Study in Rats V. 51315-049 #51859

10. Rohm & Haas #84R-082A/B, 1984; RH-53,866: Acute Definitive Eye Irritation Study in Rabbits; V. 51315-048 #51848

11. Rohm & Haas #84R-082A/B, 1984; RH-53,866: Acute Definitive Skin Irritation Study in Rabbits; V. 51315-048 #51847

12. Hazleton, 1987; RH-3,866 40WP: Delayed Contact Hypersensitivity in Guinea Pigs; V. 51315-104 #65087

13. Rohm & Haas, 1984; Material Balance and Metabolism Study of $^{14}$C-RH-3866 in Rats, Report.#310-84-16 V. 51315-083 #51894

14. Rohm & Haas, 1986; RH-3866 Kinetic and Metabolism Study in Rats Report.#83R-144-16; V. 51315-084 #51895

15. Rohm & Haas #83R-144, 1986; RH-3866 Kinetic and Metabolism Study in Mice V. 51315-085 #51896

16. Rohm & Haas #83R-204, 1984; RH-3866: A Three-month Dietary Study in Rats V.51315-054-5 #51864-5

17. Rohm & Haas #83R-204, 1984; RH-3866: A Three-month Dietary Study in Dogs Report 83R-204 V. 51315-052 #51862

18. Rohm & Haas #83R-136, 1986; RH-3866: A Three-month Dietary Study in Mice (10/8/86, V. 51315-056 #51866

19. Rohm and Haas, 1986; 4-week dermal toxicity study in rats (RH-3866; 2EC and RH-3866, 40 WP); V.51315-057; #51867;

20. Tegeris #8342, 1986; Chronic Toxicity and Oncogenicity Study with RH-3866 in Rats". V. 51315-**058-064** # **51868-74**

21. Rohm & Haas, 1986; RH-3866: One Year Dietary Study in Beagle Dogs; CDFA Vol. 51315-072-3 Rec. #51882-3

22. Rohm & Haas, 1986; Dietary Chronic and Oncogenicity Study in Mice Report # 84R-023; CDFA Vol. 51315-065-071 Rec. #51875-81

23. (Rohm & Haas, 1985; RH-53,866: Two-generation Reproduction Study in Rats CDFA Vol. 51315-078 Rec. # 51888 )

24. Rohm & Haas, 1984; Teratology Study with RH-53,866 in Rats.CDFA Vol. CDFA Vol. 51315-077 #**51887**

25. Rohm & Haas, 1984; Teratology Study with RH-53,866 in Rabbits; CDFA Vol. 51315-075 #51885

26. Imperial Chemical Industries, 1986; Triazole alanine, two-generation study in the rat. CDFA Vol. 51315-010 - 012 #49706 - 49708

27. Rohm and Haas, 1983; Microbial Mutagen Test. CDFA Vol. 51315-104 #64990

28. Rohm and Haas, 1983; Microbial Mutagen Test."CDFA Vol. 51315-104, #64991, 64992

29. Rohm and Haas, 1984; Microbial Mutagen Test - Ames Salmonella CDFA Vol. 51315-079 Rec. #50948

30. Rohm and Haas, 1984, #84R-046) RH-53,866 Technical CHO/HGPRT Gene Mutation Assay; CDFA Vol. 51315-079 Rec. #51889

31. Ciba-Geigy, 1986; Salmonella/mammalian Microsome Mutagenicity Test, CGA 131013 Technical; V. 51315-009 #49705

32. Ciba-Geigy Limited, Switzerland, 1986; Point Mutation Test with Chinese Hamster Cells V79 (Triazolylalanine.) V. 51315-009 #49702

33. Rohm and Haas, 1986, #86RC-0054) Dominant Lethal Study of RH-3866 Administered Orally via Gavage to Crl:COBS CD(SD) BR Male Rats CDFA Vol. 079 Rec. #51893

34. Rohm & Haas, 1984, #84R-074) Chromosome in vivo cytogenetics CDFA Vol. 51315-079 Rec. #51890

35. Litton, 1985; Mutagenicity Evaluation of RH-3866 Technical in an In Vitro Cytogenetic Assay Measuring Chromosomal Aberration Frequencies in Chinese Hamster Ovary (CHO) Cells CDFA Vol. 080 Rec. #51891 (R & H #84P-489]).

36. Rohm and Haas, 1987; Report No. 87R-141, RH-3866: In vivo Cytogenetics Study in Mice; V. 51315-104 #64993

37. Ciba-Geigy, Switzerland, 1986; Micronucleus Test (Chinese Hamster) (CGA 131013 Technical; Vol. 51315-009 #49704

38. Rohm & Haas #86R-084, 1986; RH-53,866 Technical In vitro Unscheduled DNA Synthesis Assay CDFA Vol. 51315-081 Rec. #51892

39. Ciba-Geigy, Switzerland, 1986; Autoradiographic DNA Repair Test on Rat Hepatocytes (CGA 131013 Technical; V. 51315-009 #49703

APPENDIX B

California Department of Food and Agriculture
Worker Health and Safety Branch

Human Exposure Assessment

Myclobutanil
(RALLY 40W)

March 21, 1988

## GENERAL CHEMISTRY

Myclobutanil is the common chemical name for alpha-butyl-alpha-(4-chlorophenyl)-1H-1,2,4-triazole-1-propanenitrile, a systemic fungicide to be marketed under the trade name RALLY 40W. The chemical formula is $C_{15}H_{17}Cl_2N_4$, with a molecular weight of 288.8 d. Technical myclobutanil is a light yellow solid with a melting point range of 63° to 68°C and a boiling point range of 202° to 208°C. The vapor pressure is $1.6 \cdot 10^{-6}$ torr. The material is soluble in common organic solvents (ketones, esters, alcohols, etc.) but insoluble in aliphatic hydrocarbon solvents. Its solubility in water is 142 ppm at 25°C. It is stable up to 300°C. The mode of action for myclobutanil's fungicide activity is the inhibition of ergosterol biosynthesis.

## FORMULATIONS

There is currently one proposed registration for myclobutanil in California. It is a wettable powder formulation packaged in water-soluble bags called RALLY 40W. It is 40 percent active ingredient.

## PROPOSED USE

The label provided for Rally$^R$ 40 W gives instructions for application to non-specified grape types for prevention and cure of Black Rot and Powdery Mildew. No other commodities are included. Three to five ounces of product per acre (0.075 to 0.125 pounds ai) are recommended, with the higher rate for use with susceptible varieties or high disease pressure. The product is to be applied in no less than 50 gallons of water per acre. Applications may begin at pre-bloom and continue on a 14 to 21 day schedule until a maximum of 1.5 pounds of product have been applied or until reaching 14 days before harvest.

## LABEL PRECAUTIONS

The proposed label carries the Toxicity Category II signal word WARNING. Precautionary Statements and Statements of Practical Treatment appear appropriate. As presently listed, protective clothing and equipment required include goggles, mid-forearm to elbow-length chemical resistant gloves, a hat, long-sleeved shirt and long pants when mixing/loading or

applying Rally$^{R}$. The user is advised to wash thoroughly after handling and before eating or smoking. The user is advised to launder contaminated clothing separately in hot water before reuse. The proposed label also gives specific directions concerning reentry; the proposed reentry interval being until spray has dried. Label instructions specifically require written or oral warnings be given to workers who may be expected to be in a treated area. Oral warnings must include the same information that written (posted) warnings would include such as date of treatment, protective equipment requirements and emergency procedures. There are also instructions in the safe handling of water soluble pouches.

BEHAVIOR ON SKIN

Studies on technical[1,2] and formulated[3] myclobutanil found it to be no more than slightly irritating to skin, but moderately[4,5] to severely[6,7] irritating to eyes. No mortality and only mild toxicity resulted from application of 5000 mg/kg dermally[8,9,10]. A subacute dermal toxicity study[11] also was done, using doses of 1, 10 and 100 mg/kg. Rats were treated 5 days a week for 4 weeks. No systemic toxicity resulted, only local skin irritation. Delayed hypersensitivity tests were performed for the technical material[12] and both liquid[13] and dry[14] formulations; the technical material was found to be slightly or equivocally allergenic. A dermal absorption study was performed on rats[15]. Myclobutanil labeled in the benzene ring with $^{14}C$ was dissolved in formulation blank to represent the emulsifiable concentrate, and an emulsion was prepared in water at 1/400 of the concentration to represent tank mix. Four rats were exposed to each of these preparations. Sixty microliters were applied to 4 $cm^2$ of shaved skin on the back of each rat. The dose site was swabbed after six hours, and the radioactivity in the swabs was counted, but only the range of recoveries in the swabs was reported. Another group of four rats was dosed with labeled myclobutanil intravenously. Excreta were collected for 7 days and assayed for radioactivity by liquid scintillation counting.

This study was interpreted by dividing the percentage of the applied dermal dose recovered from the urine by the percentage of the intravenous dose recovered from the urine and multiplying the quotient by 100. This resulted in the figure of 30% absorption of tank mix in 6 hours, which corresponds to 76% in 24 hours. This procedure is questionable for two reasons. One is that the percentages used to calculate the ratio were percentages of the nominal dose, and recovery in each case was over 100%. The other is that although excretion of the intravenous dose apparently was complete after 7 days, appreciable amounts of labeled material were still being found in the urine of dermally dosed animals on day 7, and most probably would still have been present on day 8. The animals were sacrificed after 7 days, and their carcasses and the skin from the dose site were frozen, but no report of labeled material in skin or carcass is provided.

We conclude that the estimate of dermal absorption is too low, but data are not available to recalculate it. Consequently, we must assume 100% dermal penetration.

BIOLOGICAL DISPOSITION

Feeding studies of cows[16,17,18,19], hens[20,21], rats[22,23] and mice[24] indicated rapid and complete absorption of myclobutanil from the gut. Excretion is also rapid, generally biphasic with alpha phase half life of 2 to 5 hours and beta phase half life of 25 hours or more. Excretion is almost entirely in the urine and feces, with less than 1% recovered from eggs or milk. Residues of myclobutanil are concentrated in the liver and excreted in bile, resulting in roughly equal distribution between urine and feces.

Analysis of samples by TLC resulted in the separation of 15 metabolites. Reports of the identity of seven of them have been submitted; all of the identified metabolites are oxidation products of the butyl side chain and/or of the nitrile and conjugates of these products. The 3-hydroxy butyl metabolite has been assigned the code RH-9090; it was found to constitute 23% of the labeled material recovered from the urine of cows fed labeled myclobutanil and 36% of the label recovered from eggs of dosed hens, but only 5 to 6% of the residues in rat urine. The identified metabolites account for about 80% of the recovered residues. The large number of metabolites and varying ratios make biological monitoring difficult to interpret; but since the compound is excreted largely intact, it would seem reasonable to suppose that a procedure could be developed that would convert the various partially oxidized forms to a single identifiable species.

WORKER EXPOSURE STUDY

There is one worker exposure study available for mixer/loaders and applicators[25]. Six exposure trials were conducted, three grape vineyard sites with two trials per location. Airblast application equipment was used, drawn by an open-cab tractor. The application rate was specified as 0.125 lb.a.i./acre, with an actual average rate of 0.117 lb.a.i./acre (by tank analysis). Application and mixing/loading were done by seperate personnel. The material was formulated as a wettable powder (RALLY 40W) contained in 4 oz. water soluable packets. The mixer/loaders job was primarily filling the tank with water and dropping in the required number of packets. From experience with this packaging method as an exposure mitigation procedure, the mixer/loader exposure should be extremely low, if none of the packets rupture prematurely.

Exposure monitoring was done using the following devices/procedures:

Passive dermal dosimeters (effective area 64 $cm^2$)
Cotton gloves under work-gloves
Outer glove washing with 1:1 water/isopropanol
Face swabbing with alcohol solution
Durham & Wolfe respirator exposure pads
Urine collection (48 hours)

The workers wore long pants, long-sleeved shirts, hats, butyl rubber gloves and boots (not necessarily rubber). This constituted their protective clothing. The dermal dosimeters were located both above the protective clothing (outside the coveralls) and below all outer clothing (on either bare skin or over underwear). The dosimeters were situated such that

overlap of dosimeters was as minimal as possible. The dosimeters were 4 ply construction: gauze pad + alpha cellulose pad + aluminum foil + filter paper backing sheet. The dosimeters were located on the following body sites:

On or under hat
On top of both shoulders
At nape of neck
On "V" of chest
Forearms
Thighs
Calves/Lower legs

Light cotton gloves were worn under the rubber work-gloves. The cotton gloves were used to measure potential penetrating exposure through the rubber gloves. Isopropanol: water washes were used to dislodge residue on the rubber glove exterior to estimate bare hand exposure. Face swabbing with the same alcohol solution collected removable residues on the unprotected face. Modified respirators (Durham & Wolfe) had cones fitted over respirator dust pads to estimate maximum potential inhalation exposure.

For biological monitoring, workers were instructed to collect all urines for 48 hours after the end of the workday. Laboratory and field spike recoveries for all media other than urine were greater than 90 percent.

Work tasks were separated and timed. Application took six hours, mixing/loading took two hours. These values were used for calculation of the milligrams of exposure to RALLY per hour of work-task. Table One shows the results calculated for exposure using the the data provided by the registrant. The registrant had also calculated exposure values per work-task but their method is in slight variance from CDFA standard practice. The results from both methods are comparable.

TABLE I: Calculated dermal exposure values (ug/hour) of workers mixing/loading and applying RALLY 40W (myclobutanil) fungicide to grapes.

| | CLOTHING | |
|---|---|---|
| | **w/ gloves-hat-long sleeve** | **w/o gloves-hat-short sleeve** |
| Mixer/Loader | 2.51 | 13.05 |
| Applicator | 1.37 | 45.35 |
| Mixer/Loader/Applicator | 3.88 | 58.40 |

CDFA, WH&S, H. Fong, 1988

One mixer/loader had much higher glove wash results than all the other workers. In fact, all of that worker's exterior residue results were the highest recorded for mixer/loaders. However, that worker still had non-detectable levels of residue on the interior dosimeters. Most of the calculated interior dosimeter results for both mixer/loaders and applicators are from non-detectable level (0.0002 ug/$cm^2$) extrapolations.

Gloves provided a very high degree of protection to the workers' hands. Even with exterior glove-wash samples ranging from 7.45 to 306 ug, the cotton gloves only had from non-detected to 0.9 ug on them. The butyl rubber gloves provided at minimum an eight fold mitigation factor, with an average mitigation factor of greater than 1,000.

The difference in total dermal protective clothing (gloves, hat and long-sleeves versus none) accounted for a 5-fold decrease in exposure for mixer/loaders, a 33-fold decrease for applicators and a 15-fold decrease for mixer/loader/applicators. The mitigation measures used in this study are not unusual or unreasonable.

Inhalation exposure was very low for all workers. The highest detected inhalation exposure was for an applicator; 1.090 ug total. The average applicator inhalation exposure was 0.3648 ug/day (0.061 ug/hr) and the average mixer/loader inhalation exposure was 0.0677 ug/day (0.034 ug/hr).

Biological monitoring was conducted using urine. Both myclobutanil and its metabolite (identified as RH-9090) were measured. None of the urine samples taken over the 48 hour collection period had any detectable levels of myclobutanil or RH-9090. The limit of detection was 0.01 ppm. The registrant believes this data confirms the passive dosimetery results of low levels of exposure experienced by the workers in this study. However, according to previously cited studies, there are fifteen metabolic products of which RH-9090 is only a minor constituent in rat urine. Furthermore, parent compound (unchanged myclobutanil) is not excreted in large amounts in any of the test animals. The absence of both parent and RH-9090 does not necessarily support the registrants position and further research on metabolic fate of myclobutanil in support of biological monitoring procedures is suggested.

The Lifetime Average Daily Dosage (LADD) was calculated using the previous exposure data.

TABLE II: Lifetime Average Daily Dosage (LADD) for job tasks involved in the use of RALLY 40W (myclobutanil) in grape vineyards.

| Job Task | Daily Exposure Absorbed Dose[a] (mg/12 hr) | Daily Dosage[b] (ug/kg/day) | LADD[c] (ug/kg/d) |
|---|---|---|---|
| Applicator (no gloves/hat, short sleeves) | 0.36 | 6.6 | 0.41 |
| Applicator (gloves, hat, long sleeves) | 0.01 | 0.2 | 0.01 |
| Mixer/Loader (no gloves/hat, short sleeves) | 0.10 | 1.9 | 0.12 |
| Mixer/Loader (gloves, hat, long sleeves) | 0.02 | 0.4 | 0.02 |
| Mixer/Loader/Applicator (no gloves/hat, short sleeves) | 0.47 | 8.5 | 0.53 |
| Mixer/Loader/Applicator (gloves, hat, long sleeves) | 0.03 | 0.6 | 0.04 |

a - dermal absorptions is estimated as 100 percent; therefore, daily exposure and absorbed dose are equal
b - daily dosage is for a 54.8 kg. worker
c - work period is 40 days/year in a 40 year career of a 70 year lifetime

CDFA, WH&S, H. Fong, 1988

EXPOSURE AT REENTRY

The registrant has submitted a foliar dislodgeable residue degradation study for the evaluation of potential exposure to field workers who reenter treated vineyards for cultural activities (26). Three seedless table grape vineyards near Porterville in the San Joaquin Valley were monitored beginning in mid-June, 1987, at predetermined intervals for a period of 35 days after a fifth and final application of Rally$^{R}$ 60 DF. In each case the final application rate was 0.125 pounds of active ingredient per acre for a final total of 0.5 pounds ai to each vineyard for the season. Dilution rates and earlier application rates varied, but the season total was consistant for all three vinyards. Methods used for sample collection were modified from those of Gunther, et al.(27) and Iwata, et al. (28) and the protocol was reviewed prior to the onset of the study and found to closely follow a standardized sampling plan developed by CDFA. WH&S also observed the layout of the sample areas and collection of the presamples. After collection, the leaf samples were chilled on ice until field extraction could occur. The extracts were then frozen on dry ice and shipped. Average recovery of field fortified leaf disk samples was 104 percent.

The registrant has chosen to estimate potential dermal exposure of workers

at reentry using a transfer coefficient of 10000 $cm^2$/hour which equals the highest reported transfer coefficient in the literature. This figure was arrived at by doubling the 5000 $cm^2$/hour transfer coefficient of Zweig-Popendorf (29) due to the two leaf surfaces involved. By multiplying the transfer coefficient by the quantity of residue present at the expected time of reentry and by the number of hours of expected work per day the estimated exposure is achieved. Table III presents the exposures predicted by the registrant (at 10000 $cm^2$/hour) and calculated exposures based on the original Zweig-Popendorf factor (at 5000 $cm^2$/hour). At present, there is no known transfer coefficient that applies strictly to grapes, however, estimates range between 5000 and 30,000 $cm^2$/hour. Should the transfer factor ultimately be determined experimentally to be 30,000 $cm^2$/ hour, the Annual Average Daily Dosage would be 5 and 6 ug/kg/day for males and females, respectively, reentering at 24 hours post-application.

Table III - Potential Exposure of Male and Female Fieldworkers at Reentry 2, 24 and 72 Hours Post-Application Calculated using both 5000 and 10000 $cm^2$ per hour transfer coefficients[1]

| REENTRY TIME | Daily Exposure[2] mg/day | | Daily Abs. Dosage[3] ug/kg/day | | Days/ Season[4] | AADD ug/kg/day[5] | | LADD ug/kg/day[6] | |
|---|---|---|---|---|---|---|---|---|---|
| | 5000 | 10000 | 5000 | 10000 | | 5000 | 10000 | 5000 | 10000 |
| Male | | | | | | | | | |
| 2 hours | 0.7 | 1.4 | 10 | 20 | 60 | 2 | 3 | 1.1 | 1.7 |
| 24 hours | 0.6 | 1.2 | 9 | 17 | 60 | 2 | 3 | 1.1 | 1.7 |
| 72 hours | 0.5 | 1.1 | 7 | 16 | 60 | 1 | 3 | 0.6 | 1.7 |
| Female | | | | | | | | | |
| 2 hours | 0.7 | 1.4 | 13 | 26 | 60 | 2 | 4 | 1.1 | 2.3 |
| 24 hours | 0.6 | 1.2 | 11 | 22 | 60 | 2 | 4 | 1.1 | 2.3 |
| 72 hours | 0.5 | 1.1 | 9 | 21 | 60 | 2 | 3 | 1.1 | 1.7 |

1. The application monitored was the final of 5 serial applications occurring over a 2 month period. The season total of myclobutanil applied to each of the treated vinyards was 0.5 lbs. ai.
2. Calculated daily exposure with 90 percent clothing protection factor
3. Daily exposure/body weight (70 kg male, 54.8 kg female) x 100 percent dermal absorption x 1000
4. Days of exposure per year based on four months fieldwork at 15 days per month during the period when myclobutanil is likely to be present (in this study the applications took place over a two month period; myclobutanil levels were almost non-detectable 35 days past the last application)
5. Annual Average Daily Dosage = daily absorbed dosage x 60 days/ 365 days
6. Lifetime Average Daily Dosage = AADD x 40 working years/ 70 year lifetime

CDFA, WH&S, D. Meinders, 1988

REFERENCES


1. M. E. Murphy, Acute Definitive Skin Irritation
   registration volume 51315-099 record #52514

2. M. H. Watts, Jr. Acute Definitive Skin Irritation
   registration volume 51315-104 record #64987

3. M. E. Murphy, Acute Definitive Skin Irritation
   registration volume 51315-048 record #51847

4. M. H. Watts, Jr., Acute Definitive Eye Irritation
   registration volume 51315-048 record #51848

5. M. H. Watts, Jr., Acute Definitive Eye Irritation
   registration volume 51315-104 record #64986

6. A. S. Romanello, Acute Eye Irritation, Definitive
   registration volume 51315-048 record #51858

7. M. E. Murphy, Acute Definitive Eye Irritation
   registration volume 51315-099 record #52515

8. M. E. Murphy, Acute Definitive Dermal LD50
   registration volume 51315-048 record #51846

9. M. E. Murphy, Acute Definitive Dermal LD50
   registration volume 51315-099 record #52513

10. M. H. Watts, Jr., Acute Definitive Dermal LD50
    registration volume 51315-104 record #64985

11. R. Bonin and G. A. Hazelton, RH-3866 2EC & RH-3866 40 WP 4-Week Dermal
    Toxicity Study in Rabbits
    registration volume 51315-057 record #51867

12. R. Bonin and G. A. Hazelton, RH-3866 Technical: Delayed Contact
    Hypersensitivity Study in Guinea Pigs, Report No. 87R-035
    registration volume 51315-104 (tab 9) record #64994

13. M. E. Murphy and P. K. Chan, RH-53,866 2EC: Delayed Contact Hyper-
    sensitivity Study in Guinea Pigs, Repport No. 84R-084
    registration volume 51315-050 record #51860

14. J. A. Trotter, RH-3866 40W Delayed Contact Hypersensitivity Study
    in Guinea Pigs
    registration volume 51315-104, (tab 10) record #65087

15. L. J. Didonato and R. B. Steigerwalt, RH-3866 Dermal Absorption Study in
    Male Rats, Report No. 85R-179
    registration volume 51315-086 record #51897

16. D. G. Butterworth, $^{14}C$ RH-3866 Feeding Study in Dairy Cows, Technical
    Report No. 310-84-12
    registration volume 51315-008 record #51781

17. A. H. Jacobson, Addendum to Technical Report No. 310-84-12: Characterization of Metabolites in Urine and Milk from Cows Fed 14-C-RH-3886
registration volume 51315-009 record #51782

18. K. J. Lawman, 14-C RH-3866 Feeding Study in Cows, Technical Report No. 31H-86-13
registration volume 51315-010 record #51820

19. A. H. Jacobson, Characterization and Identification of Metabolites in Cows Fed at [sic] $^{14}C$ Mixture of RH-3886/RH-9090/RH-9089
registration volume 51315-011 record #51821

20. D. G. Butterworth, $^{14}C$ RH 3866 Feeding Study in Poultry, Technical Report No. 31H-86-16
registration volume 51315-012 record #51822

21. J. J. Martin, Disposition and Metabolism of RH-3866 and Metabolites in Laying Hens; Technical Report No. 31H-86-17
registration volume 51315-013 record #51823

22. D. R. Streelman, A Material Balance and Metabolism Study of $^{14}C$ RH-3866 in Rats, Technical Report No. 310-84-16
registration volume 51315-083 record #51894

23. R. B. Steigerwalt, J. R. Udinsky and S. L. Longacre, RH-3866 Kinetic and Metabolism Study in Rats; Report No. 83R-144
registration volume 51315-084 record #51895

24. R. B. Steigerwalt, J. R. Udinsky and S. L. Longacre, RH-3866 Kinetic and Metabolism Study in Mice, Report No. 83R-175
registration volume 51315-085 record #51896

25 Rohm & Haas; Zogorski, W.J. III: Applicator Exposure Study of Myclobutanil on Grapes Tech. Report 31S-87-14
registration volume 51315-106 record #65091

26. Rohm and Haas. Determination of Dislodgeable Residues of Myclobutanil on Grape Foliage. 1987. CDFA Vol. 51315-105.

27. Gunther, et al., Establishing Dislodgeable Pesticide Residues on Leaf Surfaces. Bull. Environ. Contam. Toxicol. 9. 1973.

28. Iwata, et al., Worker Reentry Into Pesticide Treated Crops. I. Procedure for the Determination of Dislodgeable Pesticide Residues on Foliage. Bull. Environ. Contam. Toxicol. 18. 1977.

29. Zweig, et al., Journ. Agric. Food Chem. 32:1232. 1984.